

Phytochemical screening and the antioxidant, antibacterial and antifungal activities of aqueous extracts from the leaves of *Lippia triphylla* planted in Morocco

I. MALIKI^{(a)*}, A. M. ALMEHDI^(b), A. EL MOUSSAOUT^(c), I. ABDEL-RAHMAN^(b), A. OUAHBI^(a)

^(a) Laboratory of Health and Environment, Department of Biology, Faculty of Sciences Meknes, University My Ismail Meknes, Morocco.

^(b) Department of Chemistry, College of Sciences, University of Sharjah PO Box: 27272, UAE

^(c) Laboratory of Biotechnology and preservation of natural resource, faculty of science Dhar El mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco;

Abstract

The principal objective of the present study is the qualitative and quantitative evaluation of phytochemical compounds, as well as the antibacterial, antifungal and antioxidant activities of the aqueous extract of *Lippia triphylla* planted in Morocco. The phytochemical screening revealed the presence of flavonoids and alkaloids with different concentrations as well as tannins with a higher content (0.27 ± 0.02 mg. Cat/ g extract). The antioxidant activity is carried out by the following methods: the DPPH test ($IC_{50} = 9.4 \mu\text{g/ml}$ higher than BHT ($0.12 \mu\text{g/ml}$)); the β -carotene decoloration test (the inhibition of oxidation of the aqueous extract (66.17%) was important than the negative control (13. 87%)); the reducing power test (FRAP) (detection of a low iron reducing power for the aqueous extract compared to Quercetin) and the total antioxidant capacity (CAT) (this test revealed an antioxidant capacity of 935.1 ± 6.7 mg/g). On the other hand, the crude aqueous extract from the leaves of *Lippia triphylla* showed varying degrees of antimicrobial and antifungal activity against four pathogenic bacteria and two yeasts by the agar diffusion method and the direct contact method. Minimum inhibitory concentration (MIC) values for *E. coli* 57, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. cerevisiae* and *C. albicans* were 50.71 ± 5.42 , 50.23 ± 7.247 , 50 ± 2.04 , 12.5 ± 0.517 , 25 ± 0.25 and 12.5 ± 0.25 mg/ml respectively. Antimicrobial activity related to standard antibiotics was determined as a positive control.

* Corresponding author:
imanemaliki16@gmail.com

Received 28 Jun 2020,

Revised 03 Sept 2020,

Accepted 21 Sept 2019

Keywords: *Lippia triphylla*; phytochemical; screening; antifungal activity; antimicrobial activity; aromatic and medicinal plant.

1. Introduction

Lippia tryphilla is commonly named in Moroccan dialect Arabic “louiza” is a perennial a sub-shrub plant of Verbenaceae family [1] original of South America then introduced and cultivated in Mediterranean area (North Africa and Southern Europe) [2]. Genus *Lippia* contains around 250 species, herbs and shrubs traditionally used like a gastrointestinal and respiratory remedies for their various extracts with various properties antioxidant, antimicrobial, antibacterial, antifungal, larvicidal, and insecticidal activities [3]. It's can also be used as a seasoning in certain culinary preparations [4]. The aerial parts and flowers are the most used parts of this plant as an infusion utilized in morocco as a sedative and relaxing remedy in stress, anxiety and poor sleep, mainly in the newborn[1]. Despite the large consumption of *lippia*, its qualitative and quantitative composition in polyphenols is still poorly known [5]. while most recent research studies have focused on the activities of essential oil, however, advertisements on extracts have been afewer publications. [1], [3]. For this reason, the aims of the present studies were to ameliorate understanding of pharmacological activities: antioxidant, anti-bacterial, and toxicological activities and to determinate different bioactive compounds of *lippia tryphilla*..

1. Materials and methods

1.1. Plant material

Lippia tryphilla was harvested fresh from local farmers in the region of Haj Kadour in Meknes (33° 53' 42", 5° 33' 17") in northwest Morocco during the growing season March-April 2018. The identification of the species was done by the botanical professor of the Faculty of Sciences, Biology Department of Fez-Morocco. In the present study, the part of the leaves of the plant was used to prepare the preparation for extraction.

1.2. Preparation of aqueous extract

After harvesting, the plant material is cleaned and then washed with water to ensure that the plant is well preserved. It is then dried in an oven at a temperature of 35°C and in the dark for a few days. The material is then crushed by an electric grinder and stored in boxes. The aqueous extract is prepared by decoction for 30 minutes and each with three repetitions. After filtration on filter paper, the filtrate is concentrated in an oven at a temperature of 35°C.

1.3. Phytochemical screening

The screening stage considers among the preliminary studies, for this reason we have carried out a photochemical screening for the purpose of determining the phytochemical families of the selected plant, we have highlighted the following metabolites: flavonoids, tannins, terpenes, coumarins, cyanogenic compounds, alkaloids, quinones and saponins [6].

1.3.1 Determination of total polyphenols

The quantification of total phenolic compounds is carried out according to the Singleton method with Folin-Ciocalteu reagent. 500 µl of diluted F-C reagent (10/100) is added to 100µl of methanolic extract. After a few minutes, 400µl of a sodium carbonate solution (75 mg/ml) is added to the reaction medium. After incubation in the dark and at room temperature for 120 minutes, the absorbance is measured at 765 nm. The results obtained are expressed in µg gallic acid equivalent per milligram of dry extract (µg A.G.Eq/mg extract) using the linear regression equation of the calibration curve plotted for gallic acid [7].

1.3.2 Dosage of condensed tannins

The content of tannins in the deferents extracted from the studied plant is determined according to the method of vanillin in acid medium [8]: The vanillin reagent was prepared by mixing at equal volume the following dilutions: 8% HCl (v/v), 37% methanol (v/v) and 4% vanillin in methanol (w/v). The mixture was incubated at 30°C within a few minutes prior to the assay. 200 µl of each extract analysed was added to 1 ml of vanillin reagent and incubated in the dark at 30°C for 20 min. The absorbance is measured at 500 nm by a spectrophotometer against a blank consisting of a mixture of equal volume of methanol (37%) and HCl (8%). The results are expressed in µg catechol equivalent/mg dry extract with reference to the catechol calibration curve.

1.3.3 Determination of flavonoids

The determination of total flavonoids was carried out according to the method described by Dehpeur [9]: 500 µl of each extract to be analysed is added to 1500 µl 95 % methanol, 100 µl 10 % (w/v) AlCl₃, 100 µl 1 M sodium acetate and 2,8 ml distilled water. The mixture is stirred and incubated in the dark and at room temperature for 30 min. The blank is made by replacing the extract with 95 % methanol. The absorbance is measured at 415 nm using a spectrophotometer. The results are expressed in mg quercetin equivalent/g dry plant matter with reference to the quercetin calibration curve.

1.3.4 Anti-oxidant activity of the extracts studied

The antioxidant power of the two extracts tested was evaluated in vitro using four tests, the DPPH-, Reducing Power Test (FRAP), Total Antioxidant Capacity (CAT) and the β-carotene discolouration test.

1.3.4.1 Free radical scavenging DPPH- test

The DPPH test is performed following the method described by Bektaş in 2005 [10], [11]. Where 100µl of each of the methanolic solutions of the tested extracts are mixed with 750 µl of a methanolic solution of DPPH (0.004%). After incubation at room temperature for 30 minutes, the absorbance is measured at 517nm. For the negative control the sample is replaced by methanol. The results are expressed as the percentage inhibition of DPPH·, this percentage is calculated according to the following formula:

$$PI(\%) = (A_0 - A / A_0) * 100$$

PI: Percent inhibition; A₀: Absorbance of the DPPH solution without the sample (negative control); A: Absorbance of the DPPH solution in the presence of the sample.

1.3.4.2 Reductive Power Test

This test was performed according to the Moattar method in 2016 [12], [13]; 500 µl of a phosphate buffer solution (0.2 M - pH = 6.6) and 500 µl of potassium ferricyanide [K₃Fe(CN)₆] (1%) are added to 100 µl of different concentrations of the prepared samples in methanol. After incubation for 20 minutes in the water bath at 50°C; 500 µl of a 10% aqueous TCA solution, 500 µl of distilled water and 100 µl FeCl₃ of 0.1% are added to the reaction medium. Then the absorbance is determined at 700 nm against a blank containing all reagents in the absence of the test sample. The results are expressed as 50% effective concentration (EC₅₀) which reflects the concentration of antioxidant necessary to obtain an absorbance of 0.5 nm.

1.3.4.3 Total antioxidant capacity test

25 µl of each extract was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 1h30min. Then

the absorbance was measured by the spectrophotometer at 695 nm with a blank containing 25 µl methanol instead of the extract, after cooling to room temperature[14]. The total antioxidant capacity was expressed in milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g extract) from an ascorbic acid calibration curve.

1.3.4.4 *β-carotene discoloration test*

One ml of solution of β-carotene in chloroform (20 mg/100 ml) was placed in a vial, which contained 0.01 ml of linoleic acid and 100 ml of Tween 80. The chloroform was evaporated at 45 °C for 5 minutes by a vacuum rotary evaporator. 25 ml hydrogen peroxide was added to the residue. Water was added slowly to the mixture, which was vigorously stirred to form an emulsion [15], [16]. To 2.5 ml of the previous mixture, 0.1 ml of each of the extracts diluted in methanol was added in tubes. After incubating the tubes in a 50°C water bath for 120 minutes, absorbance was measured at 470 nm. The percent antioxidant activity was calculated from the following equation. A blank containing the stock solution without β-carotene was used to bring the spectrophotometer to zero. The percentage of antioxidant activity was cored with the BHT standard which was used under the same conditions for the extracts.

$$AA\% = (A_E/A_{BHT}) * 100$$

AA%: percentage of antioxidant activity; AE: absorbance after 120 min with negative control; ABHT: absorbance after 120 min of BHT.

1.3.5 Antimicrobial activity

1.3.5.1 Culture mediums

The agar media used in this work for antimicrobial activity are: Muller Hinton Agar (MHA) and Sabouraud (SB). And for the determination of the Minimum Concentration, the media used are: Malt Extract Broth (EM) and Muller Hinton Broth (MHB). The media were sterilized in an autoclave at 120°C for 20 min [17].

1.3.5.2 Biological material

Target strains; gram-negative bacteria *Escherichia coli* (ATB:57) B6N (CHU, Fez), *Escherichia coli* (ATB: 97) GMB (CHU, Fez), *Klebsiella pneumoniae* (LM, FMP, Fez), and *Pseudomonas aeruginosa* (LM, FMP, Fez); Gram-positive bacteria: *Staphylococcus aureus* (LM, FMP, Fez); Yeasts: *Candida albicans* and *Saccharomyces cerevesea*.

1.3.5.3 Products used

In this work we tested the antimicrobial activity of the aqueous extract of *Lippia tryphilla*. Positive controls: Antibiotics including Ampicillin (AMP) and Streptomycin (STR), Antifungal: Fluconazole (AF).

1.3.5.4 Preparation of the inoculum

The microbial inoculum was prepared by the direct suspension method from 2 to 3 colonies of a fresh culture which were taken aseptically, and were suspended in a solution of sterile physiological water (PES; with 0.85 to 0.9 NaCl), after vortex agitation, the turbidity was adjusted to 0.5 McFarland. The bacterial suspensions contain approximately 1x10⁸ to 2x10⁸ CFU/ml, while the yeast suspension contains approximately 1x10⁶ to 5x10⁶ CFU/ml. The McFarland standard was prepared with a mixture of 99.5 ml of a 0.36N sulphuric acid (H₂SO₄) solution with 0.5 ml of a 0.048 M dehydrated barium chloride (BaCl₂*2H₂O) solution. The absorbance of the solution was checked using a UV-Visible spectrophotometer at λ= 625nm which should be between 0.08 and 0.12. This solution was poured into 10 ml test tubes. After preparation of the inoculum, the plates containing the agar medium (MHB and SB) were inoculated with 1 ml of the bacterial and fungal suspension by the flooding technique and thus the inoculum was dispersed over the

entire agar surface (circular and back and forth movement) with removal of the excess inoculum using the propette. Finally, the boxes were dried for 10 min [18].

1.3.5.5 Disc broadcast technique

This technique is used to assess the susceptibility of microorganisms to an antimicrobial agent. After 10 min drying of the cans, sterile discs of 6 mm diameter were deposited on the surface of the agar of the previously inoculated cans and impregnated with 10 µl of test material; MPA 1.67 mg/disc, STR 0.02 mg/disc, plant extract. The concentration of AF is 5 mg/disc. Finally, the boxes were incubated at 37°C for bacteria and 30°C for yeast for 24 h. After incubation, the antimicrobial power was evaluated by measuring the growth inhibition zones in mm [19].

1.3.5.6 Microdilution technique

First the preparation of the various dilutions was carried out, firstly the dilution of 1/1000 of 0.5 McFarland microbial inoculum in the MH medium for bacteria and EM for yeast. These dilutions were further diluted ½ after inoculation of the microplate. Then stock solutions (prepared in sterile distilled water) of MPA (1.66 mg/ml) and STR (5 mg/ml), thus 5 mg/ml for FA were diluted 1/10 in MH for MPA and STR and EM medium for FA.

2. Results and discussions

2.1. Phytochemical screening

Like all plant species and specifically medicinal plants, this plant has characterized its richness in phytochemical compounds. In Table 1, phytochemical screening revealed the presence of tannins, flavonoids and alkaloids. Screening was the first step in this work for research on bioactive molecules.

Table 1: Phytochemical families presented in the studied extract

	Plant extract
Tanin	+++
Catechin tannins	-
Gallic tanins	++
Flavonoids	++ (flavonol+flavanol+genine)
Stérol	+++
Alcaloids Dragondorf test	++
Mayer test	+
Saponosides	++
Cardiac glycosides	+++
Oses ans holosides	++
Mucilage	-

+++ : Strong presence; ++ : Medium presence; + : Weak presence; - : Absent

2.2. Determination of Phenolic compounds

The quantification of polyphenols was done according to a linear calibration curve. $Y = 0.0425X + 0.2218$ and $R^2 = 0.985$ performed by gallic acid at different concentrations under the same sample conditions. The results are expressed in mg equivalent of gallic acid per 1g of extract and the total polyphenol content of the extract for the species studied is summarised in Table 4 which shows the concentrations of total polyphenols in the two species in the respective

extracts (aqueous and hydroalcoholic). The determination of the concentrations of flavonoid compounds was made according to a linear calibration curve ($Y = 0.006X - 0.0048$ and $R^2 = 0.9863$) performed by quercetin at different concentrations under the same operating conditions. The results are expressed in mg quercetin equivalent per 1 g of extract. The quantification of the tannin content in the extracts was done according to a linear calibration curve ($Y = 0.6042X + 0.065$ and $R^2 = 0.9942$) performed by catechol under the same sample conditions. The results of the quantification of these compounds revealed a concentration of total polyphenols of the order of 14.69 ± 0.76 mg equivalent of gallic acid in one gram of extract. Followed by flavonoid compounds with a concentration of the order of 9.71 ± 1.25 mg quercetin equivalent present in one gram of extract of the plant studied, Table 2. And the content of tannins remains higher with 0.27 ± 0.02 mg E.Cat/g of extract, the decoction is more effective for the extraction of tannins because it needs a temperature that facilitates the solubility and release of compounds and the extraction of phytochemical compounds exists in the material of plant origin. Thus, increasing the temperature in decoction extraction can decrease the content of total polyphenols [6].

Table 2: Concentration of phytochemical compounds measured

	Total polyphenols (mg E.AG/g)	Flavonoids (mg E.Q/g)	Tanins (μ g E.Cat/g)
Concentration	$14,69 \pm 0,76$	$9,71 \pm 1,25$	$0,27 \pm 0,02$

2.3. Anti-oxidant activity of the extracts

2.3.1 DPPH Test

The antiradical activity of the extract of this species was evaluated by the scanning ability of the free radical DPPH, Figure 1A. The results of the DPPH percent radical inhibition test and the IC-50 are recorded in Figure 1B. The IC-50 is inversely related to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the concentration of the free radical by 50%. The lower the IC-50 value, the greater the antioxidant activity of a compound [20]. Thus, according to the results obtained, we notice that the percentage of free radical inhibition for the extract is lower than that of the BHT for all the concentrations used. The anti-radical activity of the leaf extract ($IC_{50} = 9.4 \mu\text{g/ml}$), the latter remains lower compared to the BHT ($IC_{50} = 0.12 \mu\text{g/ml}$), on the other hand that of the root extract is closer to the antioxidant activity of the BHT.

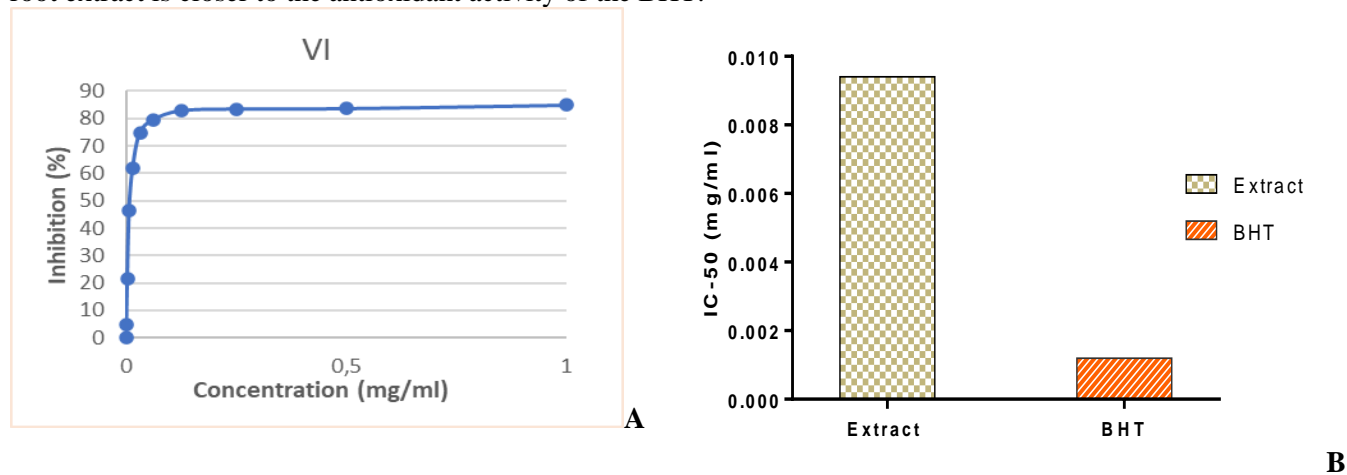


Figure 1: Antioxidant activity by the DPPH test for the aqueous extract, A: percentage inhibition as a function of the extract concentration, B: the inhibitory concentration of half of the free radicals of DPPH.

2.3.2 Decoloration of *B*-carotene

The breakdown of fatty acids is one of the main causes of food spoilage. The inhibition of the oxidation of fatty acids through the use of natural preservatives is important in the food industry. In this test the oxidation inhibition of linoleic acid is measured in the presence of β -carotene, which is used as a marker.

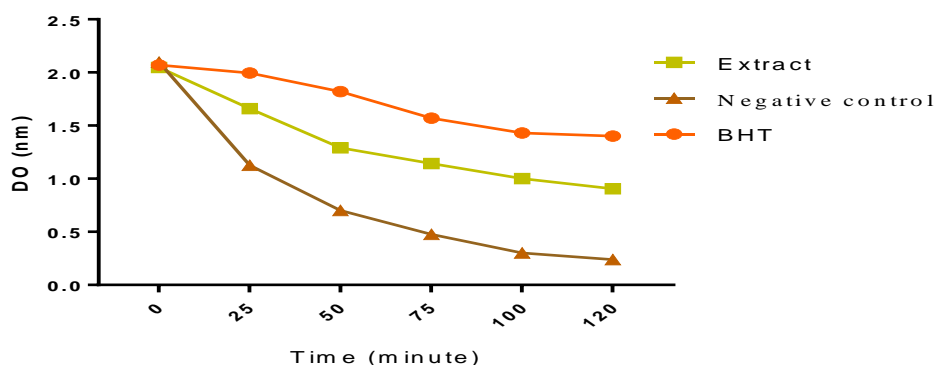


Figure 2: Evaluation of antioxidant activity by the B-carotene decoloration method.

We notice that the absorbance of β -carotene in the presence of extract, BHT and negative control decreases progressively, this decrease is important at the beginning whereas after 100 min it becomes very low. The decrease of the negative control OD is the most important followed by the leaf extract then the root extract and finally the BHT. This change in absorbance at different time intervals shows that no extracts seem to be effective as an oxidation inhibitor of linoleic acid, fig.2.

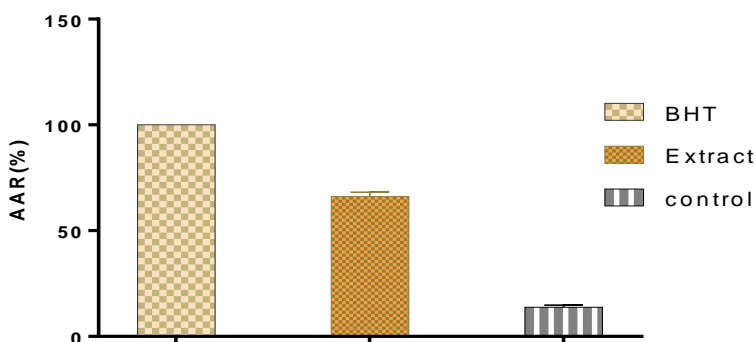


Figure 3: Anti-radical activity of extract, BHT and control in B-carotene discoloration.

It can be seen that oxidation inhibition was more important for the aqueous extract (66.17%) than the negative control (13.87%), so the antioxidant activity of both samples remains less important than the antioxidant of the positive BHT control used as reference, fig.3. The antioxidant activity of the studied extracts is confirmed by the four methods used. Through the literature search, very large differences of views are noted about the correlation between the content of total polyphenols and antioxidant power, those who have pushed scientific research further to the molecular structure of phytochemical compounds to know the reaction mechanism of certain phytochemical compounds precisely the phenolic content. Some works have shown a good correlation between IC50s and the content of polyphenols and flavonoids, while other studies have not established this correlation [21], [22] Moreover, it is well established that antioxidant activity is positively correlated with the structure of polyphenols. Generally, polyphenols with a high number of hydroxyl groups have the highest antioxidant activity [20] due to their ability to give more atoms to stabilize free radicals [23]. This may explain in part that the anti-free radical activity is dependent on the number, position and nature of substituents on the B and C rings (hydroxyl groups, metaxyl groups, glycosylated groups) and

the degree of polymerization [24]. Thus, the antioxidant effect is not only dose-dependent but also structure-dependent [25].

2.3.3 Reducing power

According to these results in Figure 4, the extract has a significantly lower antioxidant activity than the reference used (Quercetin). Metal ions are necessary for the functioning of biochemical and physiological cellular processes, but in some cases and when their mechanism of action is not well controlled, these same ions can cause lipid peroxidation, oxidative stress or tissue injury [26], [27]. For the FRAP method, the revelation of the reducing power is based on the change of the yellow colour of potassium ferrocyanide to a greenish-blue colour, the intensity of which depends on the reducing power of each sample. The intensity of the latter depends essentially on the amount of reducing power present in the test medium. This results in an increase in absorbance, which is measured at 700 nm.

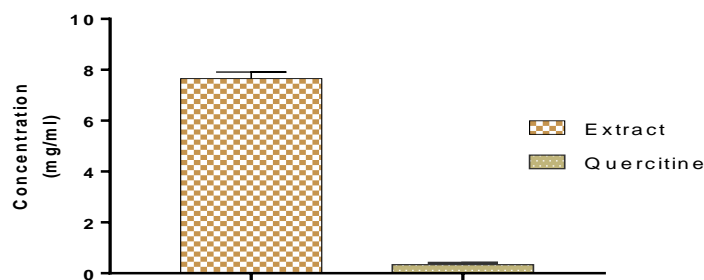


Figure 4: Antioxidant activity by the FRAP method

2.3.4 Total antioxidant capacity

Ascorbic acid was used as a standard at various concentrations. The total antioxidant capacity content is expressed in milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g EXT). The total antioxidant capacity of the extract was obtained from an ascorbic acid calibration curve. The results of this test revealed an antioxidant capacity of the order of 935.1 ± 6.7 mg/g. Many publications have shown that there is a link between antioxidant activities and the reduction power of phytochemical components in some plant species [28]. The presence of hydroxyqwl groups in phenolic compounds in plant extracts gives them the reducing power and they can serve as electron donors. For this reason, antioxidants are considered to be reducing and inactivating oxidants [29]. The autoxidation mechanism of the plant extract depends on certain parameters such as temperature, concentration of metal ion and polyphenol, pH and the presence of complexing agents [30]. Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity.

2.4. Antibacterial activity

The antibacterial activity of the aqueous extract of *L. triphylla* is evaluated qualitatively by the agar diffusion method (Table 3) and quantitatively by the microdilution method to measure the minimum inhibitory concentration of the extract under study (Table 4). The results obtained are expressed by measuring the diameter of the inhibition zone around the disc used. The results in Table 3 show that the aqueous extract has an effect on 57-gram negative *Escherichia coli* bacteria with an inhibition diameter of the order of 10 ± 0.5 mm. While the results show no other inhibition whatsoever on *K.pneumoniae* and *P.aeruginosa* which are gram-negative bacteria, or on the selected gram-positive strain (*Staphylococcus aureus*). The selected bacterial strains were resistant to the antibiotics used as reference product except for *S.aureus* which is sensitive to Streptomycin (9 ± 0.25 mm), Table 3.

Table 3: Results of the diameter of the studied extract inhibition zone and antibiotics.

	Gram-negative bacteria			Gram-positive bacteria
	<i>E.coli</i> 57	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>
Extrait	10±0.5	-	-	-
Streptomycine	-	-	-	9±0.25
Ampicilline	-	-	-	-

The minimum inhibitory concentration (MIC) of the aqueous extract of *L. triphylla* obtained is approximately the same in all the gram-negative bacterial strains used, which is of the order of 50 mg/ml, whereas the inhibitory concentration obtained in the gram-positive *S. aureus* strain remains lower than in the gram-positive bacteria with a concentration of 12.5±0.517 mg/ml. In general, however, the ICD remains accentuated compared with the reference antibiotic (Table 4).

Table 4: The minimum inhibitory concentration obtained by the micro-dilution method.

	Gram-negative bacteria			Gram-positive bacteria
	<i>E. coli</i> 57	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>
Extrait	50.71±5.42	50.23±7.247	50±2.04	12.5±0.517
Streptomycine	0.25±0.001	0.003±0.0001	-	0.062±0.003
Ampicilline	-	-	-	-

Similar results were found by [31], who studied the antibacterial activity of aqueous and organic extracts of the aerial parts of *Lippia citriodora* on Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*) bacteria; the results showed no inhibition of the aqueous extract on all the bacterial strains used, whereas the methanol and ethanol extracts showed moderate activities (the diameters of the inhibition zones are between 10. 0±0.2 mm and 32 ± 01mm), while slight growth inhibition was observed with chloroform, diethyl ether and chloroform-methanol extracts (3:1) against all bacterial species studied (inhibition zone diameters ranged from 6.2±0.2 mm to 8.4 ± 01mm). [32] tested the effect of aqueous extracts of different plants used in traditional Mexican medicine on a range of pathogenic bacteria and found that the aqueous extract of *Aloysia triphylla* had no effect on the bacteria *Escherichia coli* ATCC 25922, *Shigella flexneri*-1, *Shigella flexneri*-2, *Salmonella sp*-1 and *Salmonella sp*-2. In contrast, [33] showed that the aqueous extract of *A. triphylla* leaf showed moderate antibacterial activity on the pathogenic bacteria studied with inhibition zone diameters ranging from 9±0.0 mm to 16 ± 01 mm); it was able to inhibit *E. coli* bacteria with an inhibition zone in the range of 9 ± 0.0 mm, which is almost the same value found in the present study. The same results were noted in the study by [34] on the essential oil of *Aloysia triphylla* did not show antibacterial activity on strains of *Pseudomonas aeruginosa* (ATCC 4141) and *Enterococcus faecalis* (ATCC 2212), whereas the antibacterial activity was remarkable on the strain *Escherichia coli* (ATCC 35218) and *Bacillus subtilis* (CIP 5265) with an inhibition zone between 85±7.67 mm and 25.33±9.62 mm. The lower antibacterial activity of *Aloysia triphylla* essential oils against *Escherichia coli*, compared to that against *Bacillus subtilis*, has been attributed to the cell wall of the Gram-negative bacterium covered with an external membrane (Lipopolysaccharide, phospholipids and certain proteins); the latter prevents absorption or protects the peptidoglycan layer against the oils. Thus, the lipopolysaccharide membrane (LPS) of Gram-negative bacteria

present a permeability barrier to hydrophobic substances that can enter and inhibit Gram-positive bacteria. In Gram-positive bacteria, the peptidoglycan is outside and more in contact with the oils [34], [35]. Despite the low content of phenolic compounds in aqueous plant extracts, tests note remarkable antibacterial activity. This can be explained by the qualitative character of the chemical compounds present in the extract as much as the quantitative character, because the effectiveness of a phenolic compound depends on its physicochemical properties and its heterogeneous structure [33]. The authors suggest that the presence of different chemical agents (alkaloids, flavonoids and tannin) is responsible for the antibacterial activity of plant extracts, which explains the variations observed in the antibacterial activity of extracts from the same plant (aqueous, methanolic, ethanolic. Ct); therefore, the best efficacy of an extract is due to the synergistic action of different chemical compounds and not to a main active constituent [33], [36]. Phenolic compounds, especially flavonoids, are responsible for the antibacterial effect. Inactivation of microbial adhesins, transport proteins and cell envelope may be related to the mechanism of inhibition of hydrolytic enzymes (proteases, carbohydrates and other interactions) [37].

2.5. Antifungal activity

This part of the present study evaluated the anti-fungal effects of aqueous extracts of the leaves of *Lippia tryphilla* against two yeasts of varied metabolism: *Candida albicans* (C.a), *Saccharomyces cerevisiae* (S.c). The effects of the aqueous extracts were evaluated using the direct contact technique and the MIC technique. We report MIC to reference (control) antifungal products: Fluconazole, Cu Sulfate, Imazalil, Orthophenylphenol, Guazatin, Thiabendazole and finally the extract from our plant. According to our results, no growth of mycelial inhibition of fungi was observed around the discs containing the aqueous extract on C.albicans. On the other hand, the presence of growth inhibition is moderately observed on S. cerevisiae (S.c) with an inhibition zone of the order of 9.5 ± 1.2 mm which is larger than the zone recorded on Copper Sulphate (7.25 ± 0.4 mm) and Thiabendazole (8.51 ± 0.55 mm), comparing the values with other reference antifungal agents, but at the same time smaller than the area recorded on Imazalil (47.21 ± 3.45 mm), Guazatin (21 ± 0.55 mm) and Orthophenylphenol (17.25 ± 1.25 mm), Table 5.

Table 5: Extract inhibition zone results on fungal strains

	<i>C.albicans</i>	<i>S.Sereveseae</i>
Extract	-	9.5 ± 1.2
Fluconazole	21.2 ± 4.2	27.65 ± 2.5
Cu Sulphate	8.5 ± 0.3	7.25 ± 0.4
Imazalil	45.5 ± 0.7	47.21 ± 3.45
Orthophenylphenol	14.75 ± 0.75	17.25 ± 1.25
Guazatin	20 ± 0.78	21 ± 0.55
Thiabendazole	7.47 ± 1.5	8.51 ± 0.55

The minimum inhibitory concentration of the aqueous extract of *L. tryphilla* is generally higher than that of other reference antifungal agents. The results obtained revealed that the aqueous extract of the plant studied is effective at a concentration of 25 ± 0.25 mg/ml on *C. albicans* and at a concentration of 12.5 ± 0.25 mg/ml on *S. cerevisiae*. This value is comparable to that of copper sulphate (10 ± 0.5 mg/ml) but greater than that of the other drugs Guazatin (6 ± 0.5 mg/ml), Orthophenylphenol (1.5 ± 0.02 mg/ml), Thiabendazole (0.375 ± 0.021 mg/ml) and Imazalil (0.01 ± 0.001 mg/ml), Table 6.

Table 6: Minimum concentration of water extract inhibitor on fungal strains.

	<i>C.albicans</i>	<i>S. Sereveseae</i>
Extract	25±0.25	12.5±0.25
Fluconazole	0.4±0.02	0.2±0.01
Sulfate de Cu	10±0.25	10±0.5
Imazalil	0.05±0.007	0.01±0.001
Orthophenylphenol	1.5±0.02	1.5±0.02
Guazatin	6±0.5	6±0.5
Thiabendazole	3±0.75	0.375±0.021

This difference in the effect of the same aqueous extract on two different yeast strains regardless of the absence or average inhibition could be attributed to several causes: the yeast strains can use their own defence mechanism to neutralize its environment with appropriate techniques, therefore, it may be possible that the response threshold of each fungal species to the antifungal agent is different [38]. On the other hand, the choice of the nature and concentration of the extract studied plays a major role in the difference in results. In general, plant extracts are made up of saturated organic or aromatic compounds that have antimicrobial and antifungal properties. Ethanol or methanolic solvents are used for their initial extraction, whereas most studies avoid the use of water to extract the plant derivatives [37]. In the present study, a lower concentration of the extract was used compared to the other studies, which resulted in the absence of mycelial inhibition on *C. albicans*. The same results were recorded in an earlier study by [38] in which they investigated the effect of different concentrations of the aqueous extract of *Lippia citriodora*. No growth of mycelial inhibition was recorded while the ethanolic extract was stronger (inhibition recorded). Another study by [39] studied the antifungal activity of the ethanolic extract of the leaves of *Lippia sidoides* against 45 strains of *Candida* isolated from vaginal secretions collected from patients in Brazil and compared them with fluconazole (positive control). The results show that *Lippia sidoides* extract was active as fluconazole against most of the 45 strains of *Candida* tested. [34] in a study in 2019, evaluated the effect of essential oils of *Aloysia triphylla* L. on growth inhibition of (*Candida albicans* (ATCC 2091), *Candida kefyr*, *Candida parapsilosis*, and *Candida glabrata* (ATCC 90030)). Results showed significant inhibition of fungal growth on all four strains. In addition, analysis of the chemical composition of the essential oil showed the presence of citral, so the antifungal activity presented by the essential oils of *Aloysia triphylla* could be associated with the presence of this chemical compound. The literature highlights the fact that citral acts as a fungicidal agent because it is capable of forming a charge transfer complex with an electron donor of fungal cells, resulting in fungal death [34], [40]. Similarly, the results of the study by [41] showed complete inhibition of the growth of the *Moniliophthora roreri* strain at concentrations of 600 to 1000 µg/mL of *L. citriodora* essential oil.

3. Conclusion

the present work studies the antifungal, antimicrobial and antioxidant activity of the aqueous extract of the leaves of *lippia triphylla* planted in morocco. the results show that the aqueous extract of this plant is rich in flavonoids, alkaloids and tannins which explains the antimicrobial and antifungal activity against bacteria and standard yeasts used.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- [1] S. Makram, K. Alaoui, T. Benabbouha, B. Faridi, Y. Cherrah, A. Zellou, Extraction et activité psychotrope de l'huile essentielle de la verveine odorante *Lippia citriodora*, *Phytothérapie*. 13 (2015) 163–167. <https://doi.org/10.1007/s10298-015-0935-1>.
- [2] Z.-J. Zhang, Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders, *Life Sci*. 75 (2004) 1659–1699. <https://doi.org/10.1016/j.lfs.2004.04.014>.
- [3] E.E. Stashenko, J.R. Martínez, M.P. Cala, D.C. Durán, D. Caballero, Chromatographic and mass spectrometric characterization of essential oils and extracts from *Lippia* (Verbenaceae) aromatic plants: Gas Chromatography, *J. Sep. Science*. 36 (2013) 192–202. <https://doi.org/10.1002/jssc.201200877>.
- [4] M.E. Pascual, K. Slowing, E. Carretero, D. Sánchez Mata, A. Villar, *Lippia*: traditional uses, chemistry and pharmacology: a review, *J Ethnopharmacol*. 76 (2001) 201–214. [https://doi.org/10.1016/S0378-8741\(01\)00234-3](https://doi.org/10.1016/S0378-8741(01)00234-3).
- [5] A. Carnat, A.P. Carnat, D. Fraisse, J.L. Lamaison, The aromatic and polyphenolic composition of lemon verbena tea, *Fitoterapia*. 70 (1999) 44–49. [https://doi.org/10.1016/S0367-326X\(98\)00016-1](https://doi.org/10.1016/S0367-326X(98)00016-1).
- [6] I. Abdel-Rahman, H. Elmsellem, F. Yousfi, G. Fekkar, B. Hammouti, L. Bouyazza, R. Mohammed, M. Aiboudi, Eco-friendly *Chamaerops humilis* L. fruit extract corrosion inhibitor for mild steel in 1 M HCl, *Int. J. Corros. Scale Inhib.* 9 (2020) 446–459. <https://doi.org/10.17675/2305-6894-2020-9-2-4>.
- [7] A.E. Moussaoui, F.Z. Jawhari, D. Boust, A. Bari, PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF THE NORTHERN MOROCCAN SPECIES: WITHANIA FRUTESCENS L., *Asian Journal of Pharmaceutical and Clinical Research*. (2019) 276–279. <https://doi.org/10.22159/ajpcr.2019.v12i6.32119>.
- [8] H. Elmsellem, E. Yassir, M. Mokhtari, B. Hajar, H. Steli, A. Aouniti, A. Almehdi, I. Abdel-Rahman, H. Kusuma, B. Hammouti, A NATURAL ANTIOXIDANT AND AN ENVIRONMENTALLY FRIENDLY INHIBITOR OF MILD STEEL CORROSION: A COMMERCIAL OIL OF BASIL (*OCIMUM BASILICUM* L.), *Journal of Chemical Technology and Metallurgy*. 54 (2019).
- [9] J.A. Singleton, V.L. and Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagent, *American Journal of Enology and Viticulture*. 16 (1965) 144–158.
- [10] K. Ba, E. Tine, J. Destain, N. Cisse, P. Thonart, Comparative study of phenolic compounds, antioxidant power of various Senegalese sorghum cultivars and amylolytic enzymes of their malt, *Biotechnology, Agronomy and Society and Environment*. 14 (2010) 131–139.
- [11] A.A. Dehpour, M.A. Ebrahimzadeh, N.S. Fazel, N.S. Mohammad, Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition, *Grasas y Aceites*. 60 (2009) 405–412. <https://doi.org/10.3989/gya.010109>.
- [12] B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae), *Food Chemistry*. 90 (2005) 333–340. <https://doi.org/10.1016/j.foodchem.2003.09.013>.
- [13] A. Lahkimi, B. Louaste, I. Nechad, M. Chaouch, N. Eloutassi, Antibacterial, antifungal and antioxidant activity of *lavandula angustifolia* of the middle atlas central (Morocco), *Moroccan Journal of Chemistry*. 8 (2020). <https://revues.imist.ma/index.php/morjchem/article/view/18680> (accessed July 27, 2020).
- [14] F. Shams Moattar, R. Sariri, P. Yaghmaee, M. Giah, Enzymatic and Non-enzymatic Antioxidants of *Calamintha officinalis* Moench Extracts, *Journal of Applied Biotechnology Reports*. 3 (2016) 489–494.
- [15] M. Sabbahi, A. El-Hassouni, A. Tahani, A. El-Bachiri, Altitude effect on the chemical composition and antioxidant activity of rosemary in the region of Talsint (Morocco), *Moroccan Journal of Chemistry*. 8 (2020). <https://revues.imist.ma/index.php/morjchem/article/view/18311> (accessed July 27, 2020).

- [16] P. Maskovic, N. Manojlovic, A. Mandić, A. Mišan, I. Milovanović, M. Radojković, M. Cvijović, S. Solujić, Phytochemical screening and biological activity of extracts of plant species *Halacsya sendtneri* (Boiss.) Dörfel, *Hemijska Industrija*. 66 (2012) 43–51. <https://doi.org/10.2298/HEMIND110828068M>.
- [17] A. Chanwitheesuk, A. Teerawutgulrag, N. Rakariyatham, Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand, *Food Chemistry*. 92 (2005) 491–497. <https://doi.org/10.1016/j.foodchem.2004.07.035>.
- [18] M. Azouagh, A. Oussaid, A.I. Mansour, M. Azzouzi, M. Koudad, A. Oussaid, Contribution to the phytochemical study and evaluation of the antioxydant activities of various crudes extracts of cladodes *Opuntia ficus-indica* from the Oriental of Morocco and effect of microwave activation on drying time of the plant, *Moroccan Journal of Chemistry*. 8 (2020). <https://revues.imist.ma/index.php/morjchem/article/view/20152> (accessed July 27, 2020).
- [19] A.W. Fothergill, Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods, in: G.S. Hall (Ed.), *Interactions of Yeasts, Moulds, and Antifungal Agents: How to Detect Resistance*, Humana Press, Totowa, NJ, 2012: pp. 65–74. https://doi.org/10.1007/978-1-59745-134-5_2.
- [20] S. Saklani, A.P. Mishra, H. Chandra, M.S. Atanassova, M. Stankovic, B. Sati, M.A. Shariati, M. Nigam, M.U. Khan, S. Plygun, H. Elmsellem, H.A.R. Suleria, Comparative Evaluation of Polyphenol Contents and Antioxidant Activities between Ethanol Extracts of *Vitex negundo* and *Vitex trifolia* L. Leaves by Different Methods, *Plants* (Basel). 6 (2017). <https://doi.org/10.3390/plants6040045>.
- [21] A. El Moussaoui, F.Z. Jawhari, A.M. Almehtdi, H. Elmsellem, K. Fikri Benbrahim, D. Bousta, A. Bari, Antibacterial, antifungal and antioxidant activity of total polyphenols of *Withania frutescens* L., *Bioorg. Chem.* 93 (2019) 103337. <https://doi.org/10.1016/j.bioorg.2019.103337>.
- [22] Yassir El Ouadi, Bendif, H. Naceiri Mrabti, H. Elmsellem, Y. Kadmi, M.A. Shariati, I. Abdel-Rahman, B. Hammouti, A. Bouyanzer, Antioxidant activity of phenols and flavonoids contents of aqueous extract of *Pelargonium graveolens* origin in the North-East Morocco, *Journal of Microbiology, Biotechnology and Food Sciences*. 6 (2017) 1218–1220. <https://doi.org/10.15414/jmbfs.2017.6.5.1218-1220>.
- [23] B. Dayal, R.M. Purohit, Screening of some Indian essential oils for their antifungal properties, *Flavour Industry*. (1971). <https://agris.fao.org/agris-search/search.do?recordID=US201302260938> (accessed May 15, 2020).
- [24] Y. El Ouadi, M. Manssouri, A. Bouyanzer, L. Majidi, H. Bendaif, H. Elmsellem, M.A. Shariati, A. Melhaoui, B. Hammouti, Essential oil composition and antifungal activity of *Melissa officinalis* originating from north-Est Morocco, against postharvest phytopathogenic fungi in apples, *Microb. Pathog.* 107 (2017) 321–326. <https://doi.org/10.1016/j.micpath.2017.04.004>.
- [25] Hajar bendif, A. Melhaoui, M. Ramdani, H. Elmsellem, C. Douez, E. Yassir, Antibacterial activity and virtual screening by molecular docking of lycorine from *Pancratium foetidum* Pom (Moroccan endemic Amaryllidaceae), *Microbial Pathogenesis*. 115 (2017). <https://doi.org/10.1016/j.micpath.2017.12.037>.
- [26] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships, *The Journal of Nutritional Biochemistry*. 13 (2002) 572–584. [https://doi.org/10.1016/S0955-2863\(02\)00208-5](https://doi.org/10.1016/S0955-2863(02)00208-5).
- [27] Souad Athamena, I. Chalhém, A. Kassah-Laouar, S. Laroui, K. Souad, Activité antioxydante et antimicrobienne d'extraits de *Cuminum cyminum* L., *Lebanese Science Journal*. 11 (2010) 69–81.
- [28] A.A. Mariod, R.M. Ibrahim, M. Ismail, N. Ismail, Antioxidant activities of phenolic rich fractions (PRFs) obtained from black mahlab (*Monechma ciliatum*) and white mahlab (*Prunus mahaleb*) seedcakes, *Food Chemistry*. 118 (2010) 120–127. <https://doi.org/10.1016/j.foodchem.2009.04.085>.
- [29] A. Torres de Pinedo, P. Peñalver, J.C. Morales, Synthesis and evaluation of new phenolic-based antioxidants: Structure–activity relationship, *Food Chemistry*. 103 (2007) 55–61. <https://doi.org/10.1016/j.foodchem.2006.07.026>.
- [30] C. Popovici, I. Saykova, B. Tylkowski, Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH, *Revue de Génie Industriel*. 4 (2009) 25–39.

- [31] A. Quirós, S. Frecha-Ferreiro, A.M. Vidal-Perez, J. Lopez-Hernandez, Antioxidant compounds in edible brown seaweeds, *European Food Research and Technology*. 231 (2010) 495–498. <https://doi.org/10.1007/s00217-010-1295-6>.
- [32] M. Gulluce, F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, M. Polissiou, A. Adiguzel, H. Ozkan, Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*, *Food Chemistry*. 103 (2007) 1449–1456. <https://doi.org/10.1016/j.foodchem.2006.10.061>.
- [33] M. Öztürk, Anticholinesterase and antioxidant activities of Savoury (*Satureja thymbra* L.) with identified major terpenes of the essential oil, *Food Chemistry*. 134 (2012) 48–54. <https://doi.org/10.1016/j.foodchem.2012.02.054>.
- [34] N. Bentabet, Z. Boucherit-Otmani, K. Boucherit, Phytochemical study and evaluation of the antioxidant activity of *Fredolia aretioides* root extracts of Algeria, *Phytothérapie*. 12 (2014) 364–371. <https://doi.org/10.1007/s10298-014-0834-x>.
- [35] nabila BOUGANDOURA, nasima BENDIMERAD, Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de *Satureja calamintha* ssp. *Nepeta* (L.) Briq., *NATEC*. 5 (2013) 14–19.
- [36] N. Ghedadba, L. Hambaba, A. Ayachi, M.C. Aberkane, H. Bousselsela, S.M. Oueld-Mokhtar, Polyphénols totaux, activités antioxydante et antimicrobienne des extraits des feuilles de *Marrubium deserti* de Noé, *Phytothérapie*. 13 (2015) 118–129. <https://doi.org/10.1007/s10298-015-0944-4>.
- [37] K. N. Kiran, S.K. Kandula, R. BV, R. IB, Prof.R. Malla, R. SV, Antibacterial activity of *Lippia Citriodora* a folklore plant, *Journal of Pure and Applied Microbiology*. 2 (2008) 249–252.
- [38] A.D. Alanís, F. Calzada, J.A. Cervantes, J. Torres, G.M. Ceballos, Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders, *J Ethnopharmacol*. 100 (2005) 153–157. <https://doi.org/10.1016/j.jep.2005.02.022>.
- [39] M. Cheurfa, R. Allem, Effect of some plant extracts on pathogenic bacteria responsible for gastroenteritis, *Phytothérapie*. 15 (2017) 395–400. <https://doi.org/10.1007/s10298-017-1177-1>.
- [40] L. Rezig, M. Saada, N. Trabelsi, S. Tammar, H. Limam, I. Bettaieb, A. Smaoui, G. Sghaier, CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *ALOYSIA TRIPHYLLA* L. ESSENTIAL OILS AND METHANOLIC EXTRACT, *Italian Journal of Food Science*. 31 (2019) 17. <https://doi.org/10.14674/IJFS-1373>.
- [41] S.C. Chao, D.G. Young, C.J. Oberg, Screening for Inhibitory Activity of Essential Oils on Selected Bacteria, Fungi and Viruses, *Journal of Essential Oil Research*. 12 (2000) 639–649. <https://doi.org/10.1080/10412905.2000.9712177>.
- [42] M. Chraïbi, K. Fikri Benbrahim, H. Elmsellem, A. Farah, I. Abdel-Rahman, B. El Mahi, Y. Filali Baba, Y. kandri rodi, F. Hlimi, Antibacterial activity and corrosion inhibition of mild steel in 1.0 M hydrochloric acid solution by *M. piperita* and *M. pulegium* essential oils, *Journal of Materials and Environmental Science*. 8 (2017) 972–981.
- [43] T. Essawi, M. Srour, Screening of some Palestinian medicinal plants for antibacterial activity, *J Ethnopharmacol*. 70 (2000) 343–349. [https://doi.org/10.1016/s0378-8741\(99\)00187-7](https://doi.org/10.1016/s0378-8741(99)00187-7).
- [44] M.M. Cowan, Plant products as antimicrobial agents, *Clin. Microbiol. Rev*. 12 (1999) 564–582.
- [45] M. Ghasempour, S. Mahdavi Omran, A.A. Moghadamnia, F. Shafiee, Effect of aqueous and ethanolic extracts of *Lippia citriodora* on *Candida albicans*, *Electron Physician*. 8 (2016) 2752–2758. <https://doi.org/10.19082/2752>.
- [46] E.M.F.G. de Farias, R.M. Ximenes, L.P.M. Magalhães, A. de A. Chiappeta, K.X. da F.R. de Sena, J.F.C. de Albuquerque, Antifungal activity of *Lippia sidoides* Cham. (Verbenaceae) against clinical isolates of *Candida* species, *Journal of Herbal Medicine*. 2 (2012) 63–67. <https://doi.org/10.1016/j.hermed.2012.06.002>.
- [47] N. Kurita, M. Miyaji, R. Kurane, Y. Takahara, Antifungal Activity of Components of Essential Oils, *Agricultural and Biological Chemistry*. 45 (1981) 945–952. <https://doi.org/10.1080/00021369.1981.10864635>.
- [48] S. Lozada, L. Herrera, J. Perea, E. Stashenko, P. Escobar, In vitro effect of essential oils of three *Lippia* species on *Moniliophthora roreri* (Cif. and Par.) Evans et al., causative agent of moniliasis of cocoa (*Theobroma cacao* L.), *Acta Agronómica*. 61 (2012) 102–110.